

A1 This patent application includes a computer program listing appendix (Appendix), which contains the source code for the software used in carrying out the examples in accordance with the present invention. The Appendix is contained on one compact disc submitted in duplicate and designated as Copy 1 and Copy 2. The Appendix is in a single file that is 292 kB in size and designated "computer program listing appendix U.S. Serial No. 09-021,721". The file was created on 02/02/1998 and is a Microsoft Word document. The material in the Appendix is incorporated herein by reference.

Please replace the paragraphs beginning on page 46, line 4, to page 50, line 35 with the following paragraphs (for the convenience of the Examiner, Applicant is also including a clean copy of pages 46-50, which may be substituted for the existing pages):

GTCCAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
TCCAAAAGGGTCAGTCTACCTCCC	71.99	-1.20	SEQ ID NO: 11
CCAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO: 12
CAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20	SEQ ID NO: 13
AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20	SEQ ID NO: 14
AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20	SEQ ID NO: 15
AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20	SEQ ID NO: 16
AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20	SEQ ID NO: 17
AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20	SEQ ID NO: 18
GGGTCAGTCTACCTCCCGCCATAAA	76.85	-1.20	SEQ ID NO: 19
GTCAGTCTACCTCCCGCCATAAAAA	73.10	-0.80	SEQ ID NO: 20
TCAGTCTACCTCCCGCCATAAAAAA	69.50	0.90	SEQ ID NO: 21
CAGTCTACCTCCCGCCATAAAAAAC	65.60	0.90	SEQ ID NO: 22
AGTCTACCTCCCGCCATAAAAAACT	64.96	0.90	SEQ ID NO: 23
GTCTACCTCCCGCCATAAAAAACTC	65.48	1.10	SEQ ID NO: 24
TCTACCTCCCGCCATAAAAAACTCA	66.36	2.40	SEQ ID NO: 25
CTACCTCCCGCCATAAAAAACTCAT	64.97	2.90	SEQ ID NO: 26
TACCTCCCGCCATAAAAAACTCATG	63.96	2.70	SEQ ID NO: 27
ACCTCCCGCCATAAAAAACTCATGT	62.58	1.10	SEQ ID NO: 28
CCTCCCGCCATAAAAAACTCATGTT	65.10	0.40	SEQ ID NO: 29
CTCCCGCCATAAAAAACTCATGTTT	64.96	0.10	SEQ ID NO: 30
TCCCGCCATAAAAAACTCATGTTCA	63.37	-0.10	SEQ ID NO: 31
CCCGCCATAAAAAACTCATGTTCAA	62.86	-0.10	SEQ ID NO: 32
CCGCCATAAAAAACTCATGTTCAAG	60.47	-0.10	SEQ ID NO: 33
CGCCATAAAAAACTCATGTTCAAGA	57.98	-0.10	SEQ ID NO: 34
	56.20	-0.10	SEQ ID NO: 35

A2 Next, the oligonucleotide sequences are filtered on the basis of T_m . A high and low cut-off value may be selected, for example, $60^\circ C \leq T_m \leq 85^\circ C$. Thus, oligonucleotides having T_m values falling within the above range are retained. Those outside the range are

discarded, which is indicated below by lining out of those oligonucleotides and parameter values.

GTCCAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
TCCAAAAGGGTCAGTCTACCTCCC	71.99	-1.20	SEQ ID NO: 11
CCAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO: 12
CAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20	SEQ ID NO: 13
AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20	SEQ ID NO: 14
AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20	SEQ ID NO: 15
AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20	SEQ ID NO: 16
AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20	SEQ ID NO: 17
AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20	SEQ ID NO: 18
GGTCAGTCTACCTCCCGCCATAAAA	76.85	-1.20	SEQ ID NO: 19
GGTCAGTCTACCTCCCGCCATAAAAA	73.10	-0.80	SEQ ID NO: 20
GTCAGTCTACCTCCCGCCATAAAAAA	69.50	0.90	SEQ ID NO: 21
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
CTCCCGCCATAAAAAACTCATGTTT	63.37	-0.10	SEQ ID NO: 31
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
CCGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

Next, the oligonucleotide sequences remaining after the above exercise are filtered on the basis of ΔG_{MFOLD} and are retained if the value is greater than - 0.4. Those oligonucleotides with a ΔG_{MFOLD} less than - 0.4 are discarded, which is indicated below by double lining out of those oligonucleotides and parameter values.

GTCCAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
TCCAAAAGGGTCAGTCTACCTCC	71.99	-1.20	SEQ ID NO: 11
CCAAAAGGGTCAGTCTACCTCC	70.78	-1.20	SEQ ID NO: 12
CAAAAGGGTCAGTCTACCTCC	71.23	-1.20	SEQ ID NO: 13
AAAAAGGGTCAGTCTACCTCC	73.07	-1.20	SEQ ID NO: 14
AAAGGGTCAGTCTACCTCC	75.68	-1.20	SEQ ID NO: 15
AAGGGTCAGTCTACCTCC	77.53	-1.20	SEQ ID NO: 16
AGGGTCAGTCTACCTCC	79.03	-1.20	SEQ ID NO: 17
GGGTCAGTCTACCTCC	79.03	-1.20	SEQ ID NO: 18
GTCAGTCTACCTCC	76.85	-1.20	SEQ ID NO: 19
GTCAGTCTACCTCC	73.10	-0.80	SEQ ID NO: 20
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
CTCCCGCCATAAAAAACTCATGTTT	63.37	-0.10	SEQ ID NO: 31
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
CCCGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
CCCGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

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cont

Clusters of retained oligonucleotides are identified and ranked based on cluster size. In this example a contiguous cluster of 13 retained oligonucleotides is identified by the vertical black bar on the left. All of the oligonucleotides in this cluster may be evaluated experimentally.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	71.77	-1.20	SEQ ID NO: 10
TCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	71.99	-1.20	SEQ ID NO: 11
CCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	70.78	-1.20	SEQ ID NO: 12
CAAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	71.23	-1.20	SEQ ID NO: 13
AAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	73.07	-1.20	SEQ ID NO: 14
AAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	75.68	-1.20	SEQ ID NO: 15
AAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	77.53	-1.20	SEQ ID NO: 16
AAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	79.03	-1.20	SEQ ID NO: 17
AGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	79.03	-1.20	SEQ ID NO: 18
GGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	76.85	-1.20	SEQ ID NO: 19
GGTTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	73.10	-0.80	SEQ ID NO: 20
GTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	69.50	0.90	SEQ ID NO: 21
TCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	65.60	0.90	SEQ ID NO: 22
CAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	64.96	0.90	SEQ ID NO: 23
AGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	65.48	1.10	SEQ ID NO: 24
GTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	66.36	2.40	SEQ ID NO: 25
TCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	64.97	2.90	SEQ ID NO: 26
CTACCTCCCGCCATAAAAAACTCATGTTCAAGA	63.96	2.70	SEQ ID NO: 27
TACCTCCCGCCATAAAAAACTCATGTTCAAGA	62.58	1.10	SEQ ID NO: 28
ACCTCCCGCCATAAAAAACTCATGTTCAAGA	65.10	0.40	SEQ ID NO: 29
CCTCCCGCCATAAAAAACTCATGTTCAAGA	64.96	0.10	SEQ ID NO: 30
CTCCCGCCATAAAAAACTCATGTTCAAGA	63.37	-0.10	SEQ ID NO: 31
TCCCGCCATAAAAAACTCATGTTCAAGA	62.86	-0.10	SEQ ID NO: 32
CCCGCCATAAAAAACTCATGTTCAAGA	60.47	-0.10	SEQ ID NO: 33
CCGCCATAAAAAACTCATGTTCAAGA	57.98	-0.10	SEQ ID NO: 34
CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

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